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Establishing Vetiver Based Treatment System for Treating the Wastewater from Paperboard Mill

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Paperboard mills are abundant user of water resource and there by produce large amount of wastewater from the process of paper manufacturing. The wastewater is rich in organic matter and nutrients which could pollute the water bodies and it can also affect the crops when releasing it for irrigation. Here an attempt was made to treat and polish the paperboard mill effluent by using vetiver in hydroponics setup. The vetiver based treatment studies on quality parameters of wastewater revealed that the total nitrogen, total phosphorus, calcium, magnesium, sodium, potassium, carbonate, bicarbonate and chloride were reduced significantly. Among the treatments, a significant reduction of total nitrogen (59.24 %), total phosphorus (47.78 %), potassium (54.64 %), sodium (30.04 %), calcium (26.27 %), magnesium (40.12 %), carbonate (33.5 %), bicarbonate (12.88 %) and chloride (23.36 %) were recorded in treated effluent + aeration + vetiver system (T6). The lead and cadmium content in the wastewater were decreased significantly by 65.63 % and 64.29 % respectively in T6 (Treated effluent + Aeration + Vetiver system). The growth parameters viz., number of leaves and dry matter production were favourably influenced by the paperboard mill wastewater. The incremental growth performance of vetiver was recorded in treated effluent which was aerated. The higher proline activity was noticed in vetiver shoot grown in untreated effluent, whereas, the higher electrolyte leakage rate of vetiver shoot and root was recorded in treated effluent + aeration + vetiver system.

Key words: Vetiver system, Paperboard mill, Hydroponics, Treated effluent, Aeration, Lead and Cadmium

The water available from surface and groundwater sources are known to be clean and free from contamination, since the release of wastewater into surface results in contamination of water bodies (Morrison *et al*., 2001). Guo and Sims (2003) states that, on forecasting the waste products for years, effluent is a potential resource on various applications such as industrial and domestic reuse and also for the agricultural and recreational purposes. There are many conventional methods for remediation now a days. Although, the conventional methods of wastewater treatments are available to treat the wastewater, there is a need of environmental friendly technology to meet the environmental standards. Phyto-remediation is one of the biological wastewater treatment methods which is low cost, consumes less energy, natural, practicable, effective and simple. Suitable plant species used for phyto-remediation should have high uptake of both organic and inorganic pollutants, grow well in polluted water and be easily controlled in quantitatively propagated dispersion. Contrarily, phytoremediation can also utilized for effluent polishing for the removal of additional suspended solids and other pollutants from secondary effluent to meet the drinking water quality standards. Vetiver grass (*Vetiveria zizanioides* (L.) Nash recently reclassified as *Chrysopogon zizanioides* (L.) Roberty) belongs to the gramineae family and was first used for soil and water conservation purpose in all countries. Due to its unique morphological, physiological characteristics, the tolerance to high levels of heavy metals and adverse conditions, its role has been successfully extended to environmental protection, particularly in the field of wastewater treatment. Vetiver system for wastewater treatment is an unique innovative technology which was developed in queensland, Australia in 1995 for having a "super absorbent" characteristics. It is a green and environmental friendly wastewater treatment technology as well as a natural recycling method (i.e. in the process of 'treatment', the vetiver plant absorbs essential plant nutrients such as N, P and cations). Hence, the wastewater discharge into the lakes and water bodies will not be a serious issue, since, it has been loaded with minimum pollutant considerably. Therefore, vetiver is a superseding solution for the treatment of wastewater.

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Material and Methods

A series of experiments have been conducted with to treat the paperboard mill effluent with vetiver at Tamil Nadu Agricultural University, Coimbatore. The paper board mill effluent was collected from Indian Tobacco Company, Paperboards and Speciality Papers Division (ITC-PSPD) Thekkampatty village, near Mettupalayam in Coimbatore District of Tamil Nadu. Both raw and treated effluent samples were collected and analyzed for their physico- chemical properties.

Analysis of effluent samples

Chemical properties

The quality parameters such as total nitrogen was determined by semi automatic kjeldahl distillation (Diacid extract – preparation of diacid extract by mixing H_2SO_4: $HClO_4$ @ 5:2), total phosphorus was determined by tri acid extract. The cation such as potassium, sodium were determined by flame photometer, whereas, calcium and magnesium were determined by versenate titration method (Jackson, 1967). The anion such as carbonate and bicarbonate were determined by titrating with 0.1 N H_2SO_4 using phenolphthalein and methyl orange indicator (Piper, 1966), while the chloride determination was done by Mohr's method (Jackson, 1967).

Vetiver based treatment system

A laboratory experiment has been designed to evaluate the efficiency of vetiver based treatment system. The three months old vetiver slips propagated in the polybag were collected from the private nursery in Coimbatore. The slips were handled carefully while removing from the polybag and it washed with tap water to remove the adhered soil particles in the roots of vetiver. For acclimatization, it was exposed to tap water for one week and then the shoots of vetiver were trimmed to height of 15 cm and it was concised with 6 leaves per slip (single tiller). The vetiver roots were trimmed after 10 cm length in order to observe the growth of vetiver

Experimental setup

The laboratory scale hydroponic system has been fabricated by using high density polyethylene containers which has a capacity of 25 litres. The lids of plastic containers were drilled with 12 holes with diameter of 2.5 cm and spacing of 4.0 X 2.5 cm between holes, which serves as a raft to support vetiver. The experiment was conducted for the period of 40 days. Different treatments with untreated effluent and treated effluent of paperboard mill were imposed with trimmed vetiver grass. Six treatments were imposed with 4 replication as follows (T_1 : Untreated effluent; T_2 : Treated effluent; T_3 : Untreated effluent + Vetiver system; T_4 : Untreated effluent + Aeration + Vetiver system; T_5 : Treated effluent + Vetiver system; T_6 : Treated effluent+ Aeration+ Vetiver system). The treated effluent was collected from ETP (Effluent Treatment Plant) from the secondary clarifier. The treatments T_4 (Untreated effluent + Aeration + Vetiver system) and T_6 (Treated effluent + Aeration + Vetiver system) have been aerated using aquarium air pump motor and it was aerated continuously throughout the study period.

Each polyethylene plastic containers were filled with 20 litres of effluent. The lid of the container designed as a raft to support the vetiver to float in the container. Each hole has accompanied with a slip and it was supported with a sponge to hold the slip straight. Likewise, each container carried 12 vetiver slips.

Collection and analysis of wastewater

The water samples from the experiment were collected at 10 days interval and analysed for the chemical properties *viz.,* total nitrogen, total phosphorus, calcium, magnesium, sodium, potassium, chloride, carbonate, bicarbonate, lead and cadmium.

Biometric analysis

The leaves were counted at 40^{th} day and the increment in number of leaves was recorded. In each treatment, the shoot and root of vetiver were collected at 40^{th} day and dried it in oven at temperature of 70º celsius and it was expressed in grams.

Enzyme analysis

Proline

The fresh specimen was collected at 40^{th} day of experiment from various treatments. 0.5 g of samples was homogenized with 3% aqueous sulphosalicylic acid and filtered through whatman no.2 filter paper. Then 2 ml filtrate was taken in test tube added with 2 ml glacial acetic acid + 2 ml acid ninhydrin and heated in boiling water bath for one hour. Then, it was terminated by placing the tubes in ice bath. Addition of 4 ml of toluene to the reaction mixture and it was stirred well for 20 – 30 seconds. Afterwards, toluene layer was separated using separating funnel and measured the red colour intensity at 520 nm along with a series of standard with pure proline (Bates *et al.,* 1973).

Electrolyte leakage rate

The membrane permeability was reflected by ELR. The fresh leaves (1 gram) were cut into pieces of 5 mm length and placed in two test vials. 20 ml of deionized water was added to both the vials. One vial was incubated at 25 degree celcius on rotary shaker for 24 hrs and the EC was measured as EC1. The second vial was autoclaved at 120 degree celcius for 20 minutes to release all electrolytes and finally measured the EC as EC2 (Lutts *et al.,* 1996)

$$\text{Electrolyte leakage rate (ELR) \%} = (EC_1 / EC_2) \times 100$$

Results and Discussion

Initial Characteristics of wastewater

The effluent (untreated and treated) characteristics for the treatment with vetiver are presented in Table 1.

The total nitrogen and total phosphorus were 39 and 9.25 mg L^{-1} in untreated effluent, whereas in treated effluent 25 and 8.5 mg L^{-1}, respectively. The potassium levels of untreated and treated effluent were 30 mg L^{-1} and 18.5 mg L^{-1}.

The cations like calcium, magnesium were also present in the untreated effluent at the concentration of 440 mg L^{-1} and 145 mg L^{-1} and in the treated effluent 245 mg L^{-1} and 124 mg L^{-1}. The sodium content of untreated and treated effluent were 285 mg L^{-1}

Table 1. Initial characteristics of paperboard mill effluent

Characteristics	Untreated effluent	Treated effluent
Total nitrogen (mg L^{-1})	39.0	25.0
Total phosphorus (mg L^{-1})	9.25	8.5
Potassium (mg L^{-1})	30.0	18.5
Calcium (mg L^{-1})	440	245
Magnesium (mg L^{-1})	145	124
Sodium (mg L^{-1})	285	250
Chloride (mg L^{-1})	580	410
Carbonate (mg L^{-1})	36	28
Bicarbonate (mg L^{-1})	142.5	130
Lead (mg L^{-1})	2.01	0.96
Cadmium (mg L^{-1})	1.90	0.42

and 250 mg L^{-1}, respectively and the potassium content of untreated and treated effluent were 30 mg L^{-1} and 18.5 mg L^{-1}, respectively. The anions like chloride, carbonate, bicarbonate content in the untreated effluent were 580 mg L^{-1}, 36 mg L^{-1}, 142.5 mg L^{-1}, and in the treated effluent 410 mg L^{-1}, 28 mg L^{-1}, 130 mg L^{-1}, respectively. The lead content of untreated and treated effluent were 2.01 and 0.96 mg L^{-1}, respectively and the cadmium content of untreated and treated effluent were 1.90 and 0.42 mg L^{-1}, respectively. Both untreated and treated effluent had considerable amount of nutrients *viz.,* total nitrogen, total phosphorus, calcium, magnesium, sodium and potassium.

Effect of vetiver based treatment system on wastewater characteristics

Nitrogen and Phosphorus

The total nitrogen content of wastewater of all treatments registered between 10.19 to 38.17 mg L^{-1} (Table 2). The highest value (36.75 mg L^{-1}) was recorded in T_1 (Untreated effluent) and the least value (10.19 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The decreasing trend was followed in all the treatments at every 10 days interval, excluding the untreated and treated effluent alone. The percentage removal of nitrogen was higher (59.24 %) in T_6 (Treated effluent + Aeration + Vetiver system) followed by (Unreated effluent + Aeration + Vetiver system) recorded 57.35 %. The reduction of total nitrogen in the wastewater may be due to the assimilation by vetiver or the oxidation of ammonium into nitrite and nitrate by nitrifying bacteria (Boonsong and Chansiri, 2008)

Table 2. Effect of vetiver on Total nitrogen (mg L^{-1}) of paperboard mill wastewater

Treatments	Total nitrogen (mg L^{-1})				Mean
	10^{th} day	20^{th} day	30^{th} day	40^{th} day	
T_1 - Untreated effluent	38.50	38.26	38.05	37.85	38.17
T_2 - Treated effluent	24.50	24.28	24.12	23.98	24.22
T_3 - Untreated effluent + Vetiver system	29.75	21.00	19.25	14.00	21.00
T_4 - Untreated effluent + Aeration + Vetiver system	22.75	17.50	14.00	12.25	16.63
T_5 - Treated effluent + Vetiver system	17.50	10.50	8.75	7.50	11.06
T_6 - Treated effluent+ Aeration+ Vetiver system	15.75	9.50	8.50	7.00	10.19
Mean	24.79	20.17	18.78	17.10	
SEd	0.285	0.311	0.251	0.382	
CD (0.05)	0.598	0.654	0.528	0.803	

Table 3. Effect of vetiver on Total Phosphorus (mg L^{-1}) of paperboard mill wastewater

Treatments	Total Phosphorus (mg L^{-1})				Mean
	10^{th} day	20^{th} day	30^{th} day	40^{th} day	
T_1 - Untreated effluent	9.20	9.17	9.12	9.07	9.14
T_2 - Treated effluent	8.41	8.38	8.33	8.29	8.35
T_3 - Untreated effluent + Vetiver system	7.86	5.78	4.93	3.28	5.46
T_4 - Untreated effluent + Aeration + Vetiver system	7.50	5.65	4.03	2.96	5.04
T_5 - Treated effluent + Vetiver system	6.19	5.14	4.99	4.85	5.29
T_6 - Treated effluent+ Aeration+ Vetiver system	6.06	5.10	4.85	3.31	4.83
Mean	7.54	6.54	6.04	5.29	
SEd	0.085	0.070	0.074	0.076	
CD (0.05)	0.180	0.147	0.155	0.160	

The total phosphorus content was significantly influenced by the treatments (Table 3). Among the treatments, the maximum per cent removal of phosphorus was recorded in T_6 (Treated effluent + Aeration + Vetiver system) (47.78 %) followed by T_4 (Untreated effluent + Aeration + Vetiver system)

Table 4. Effect of vetiver on potassium content (mg L^{-1}) of paperboard mill wastewater

Treatments	Potassium (mg L^{-1})				Mean
	10th day	20th day	30th day	40th day	
T_1 - Untreated effluent	30.00	30.50	30.75	31.20	30.61
T_2 - Treated effluent	18.50	18.65	18.70	18.80	18.66
T_3 - Untreated effluent + Vetiver system	23.50	19.75	13.65	10.50	16.85
T_4 - Untreated effluent + Aeration + Vetiver system	21.28	15.75	11.25	9.50	14.45
T_5 - Treated effluent + Vetiver system	14.00	9.75	8.15	6.90	9.70
T_6 - Treated effluent+ Aeration+ Vetiver system	12.50	8.54	6.48	6.05	8.39
Mean	19.96	17.16	14.83	13.83	
SEd	0.167	0.223	0.168	0.174	
CD (0.05)	0.352	0.469	0.354	0.366	

(40.70 %). The highest value of 9.14 mg L^{-1} was recorded in T_1 (untreated effluent) and the lowest value (4.83 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The decreasing trend was observed in all treatments at every 10 days interval. The observed phosphorus removal

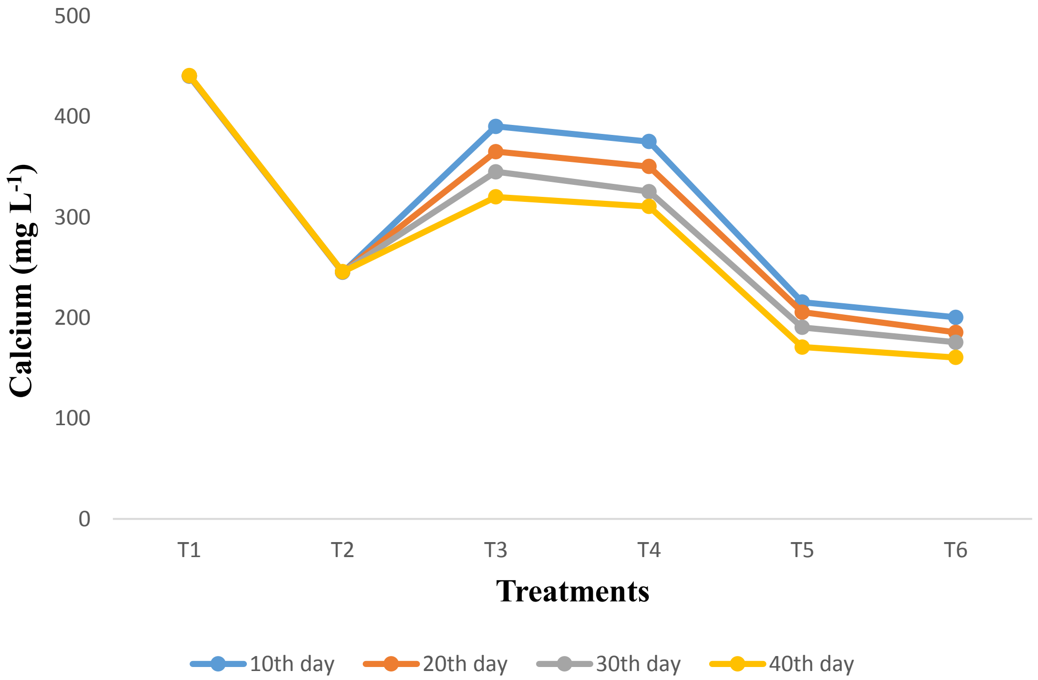

Fig. 1. Effect of vetiver on calcium (mg L^{-1}) content of paperboard mill wastewater

might be due to absorption by vetiver, sedimentation in the container and the ability of a particular group of micro-organisms (Acinetobacter) to take up and store excessive amounts of phosphate (Boonsong and chansiri, 2008)

Table 5. Effect of vetiver on sodium content (mg L^{-1}) of paperboard mill wastewater

Treatments	Sodium (mg L^{-1})				Mean
	10th day	20th day	30th day	40th day	
T_1 - Untreated effluent	285.38	285.70	285.92	285.98	285.75
T_2 - Treated effluent	252.42	252.50	252.78	252.90	252.65
T_3 - Untreated effluent + Vetiver system	260.00	245.25	230.15	218.75	238.54
T_4 - Untreated effluent + Aeration + Vetiver system	235.00	218.50	185.50	172.50	202.88
T_5 - Treated effluent + Vetiver system	205.00	190.45	179.45	165.85	185.19
T_6 - Treated effluent+ Aeration+ Vetiver system	196.00	180.15	168.72	154.75	174.91
Mean	238.97	228.76	217.09	208.46	
SEd	2.744	1.177	2.992	3.569	
CD (0.05)	5.766	2.474	6.287	7.499	

Cations

The potassium and sodium content of papermill wastewater was significantly influenced by the different treatments (Table 4&5). The potassium and sodium percentage removal were higher in T_6 (Treated effluent + Aeration + Vetiver system) followed by T_4 (Untreated effluent + Aeration + Vetiver system). The potassium content was maximum (30.61 mg L^{-1}) in T_1 (Untreated effluent) and the minimum (8.39 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The highest percentage of sodium (30.04 %) and potasium (54.65 %) removal were noticed in T_6 (Treated effluent + Aeration + Vetiver system). Similarly, maximum sodium content (285.75 mg L^{-1}) in the wastewater was observed in T_1 (Untreated effluent) and the minimum sodium content (174.91 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The highest percentage removal ranked in the order $T_6 > T_4 > T_1$.

Table 6. Effect of paperboard mill wastewater on number of leaves and dry matter production @ 40th day

Treatments	Number of leaves	Dry matter production @ 40th day	
		Shoot (g)	Root (g)
T_1 - Untreated effluent	-	-	-
T_2 - Treated effluent	-	-	-
T_3 - Untreated effluent + Vetiver system	9.00	20.00	23.50
T_4 - Untreated effluent + Aeration + Vetiver system	15.00	35.00	39.20
T_5 - Treated effluent + Vetiver system	17.00	38.50	40.70
T_6 - Treated effluent+ Aeration+ Vetiver system	20.00	42.40	45.70
Mean	15.25	33.98	37.28

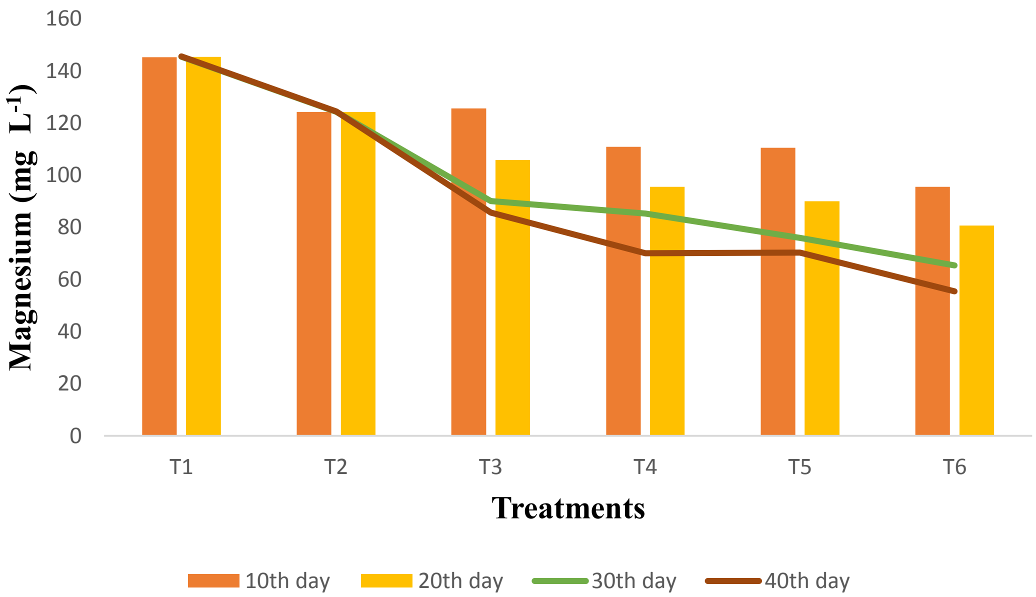

Fig 2. Effect of vetiver on magnesium (mg L^{-1}) content of paperboard mill wastewater

Table 7. Effect of paperboard mill wastewater on Proline and Electrolyte Leakage Rate of vetiver @ 40th day

Treatments	Proline (microgram $gram^{-1}$)	ELR (%)	
	Shoot	Shoot	Root
T_1 - Untreated effluent	-	-	-
T_2 - Treated effluent	-	-	-
T_3 - Untreated effluent + Vetiver system	3.49	93.04	78.81
T_4 - Untreated effluent + Aeration + Vetiver system	3.34	89.55	57.42
T_5- Treated effluent + Vetiver system	1.75	85.69	70.59
T_6 - Treated effluent+ Aeration+ Vetiver system	0.67	77.52	38.25
Mean	2.31	86.45	61.27

The calcium content was significantly influenced by various treatments (Fig. 1 & 2). The maximum value (440.65 mg L^{-1}) recorded in T_1 (Untreated effluent) and the minimum (180.63 mg L^{-1}) was recorded in T^6 (Treated effluent + Aeration + Vetiver system). Among the treatments, T_6 (Treated effluent + Aeration + Vetiver system) showed the higher percentage removal (26.27 %) followed by T_4 (Unreated effluent + Aeration + Vetiver system) recorded 22.59 % of removal. The magnesium content was found to be decreased trend in all the treatments. The highest percentage removal (40.12 %) was recorded in T_6 (Treated effluent + Aeration + Vetiver system) followed by T_4 (Untreated effluent + Aeration + Vetiver system) recorded 37.64 % of removal. The maximum value (145.40 mg L^{-1}) was noticed in T_1 (Untreated effluent) and the minimum value (74.25 mg L^{-1}) was noticed in T_6 (Treated effluent + Aeration + Vetiver system). Keshtkar *et al.* (2016) reported that vetiver removed upto 59.1 % of sodium, 58.4 % of potassium, 51.5 % of calcium, 48.7% of magnesium in unconventional wastewater.

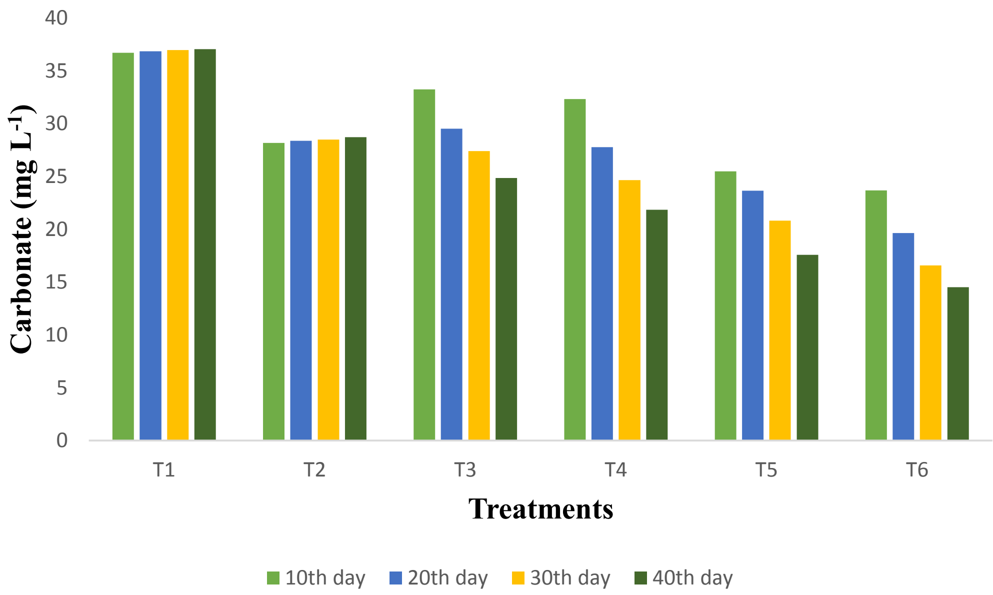

Fig 3. Effect of vetiver on carbonate (mg L^{-1}) content of paperboard mill wastewater

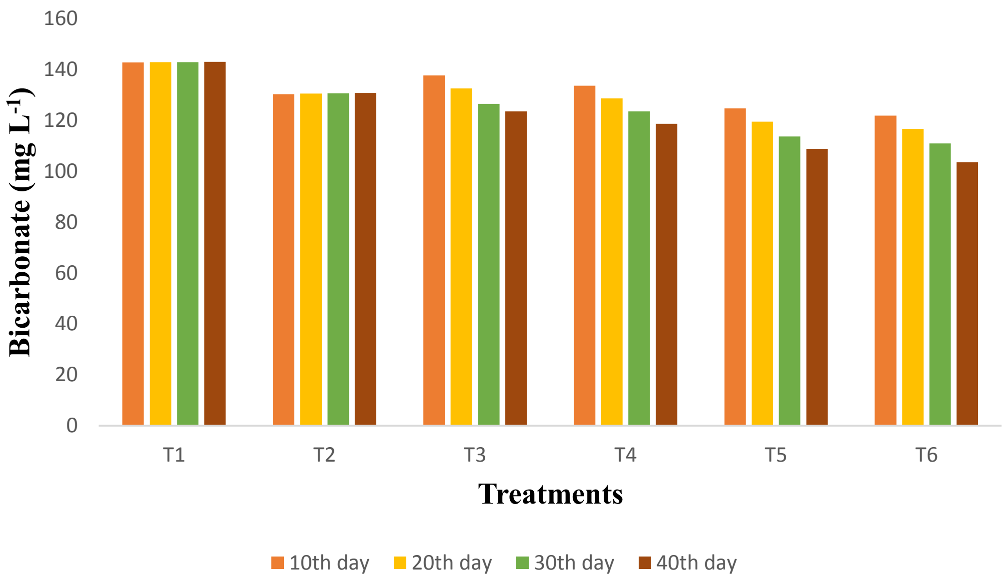

Fig 4. Effect of vetiver on bicarbonate (mg L^{-1}) content of paperboard mill wastewater

Anions

The difference on wastewater carbonate and bicarbonate at 10 days interval are presented in the fig 3 & 4. The carbonate and bicarbonate content of wastewater ranges from 36.90 to 18.62 mg L^{-1} and

142.90 to 113.25 mg L^{-1}, respectively. The decreased trend of carbonate and bicarbonate values were observed in all treatments. The effect of treatments on the wastewater was found to be significant at each 10 days interval. Among the treatments, T_6 (Treated effluent+ Aeration+ Vetiver system) recorded the lowest value of carbonate (18.62 mg L^{-1}) and bicarbonate (113.25 mg L^{-1}) and higher value of carbonate (36.90 mg L^{-1}) and bicarbonate (142.90 mg L^{-1}) were registered in T_1 (Untreated effluent). The higher percentage removal of carbonate (33.5 %) and bicarbonate (12.88 %) were recorded in treated effluent along with aeration. The removal might be due to the extensive root system of vetiver which leads to absorption of carbonate and bicarbonate for growth. This is supported by Truong and Hart (2001) who identified that vetiver removed 59 % of carbonate and bicarbonate while treating with high concentration and low concentration leachate.

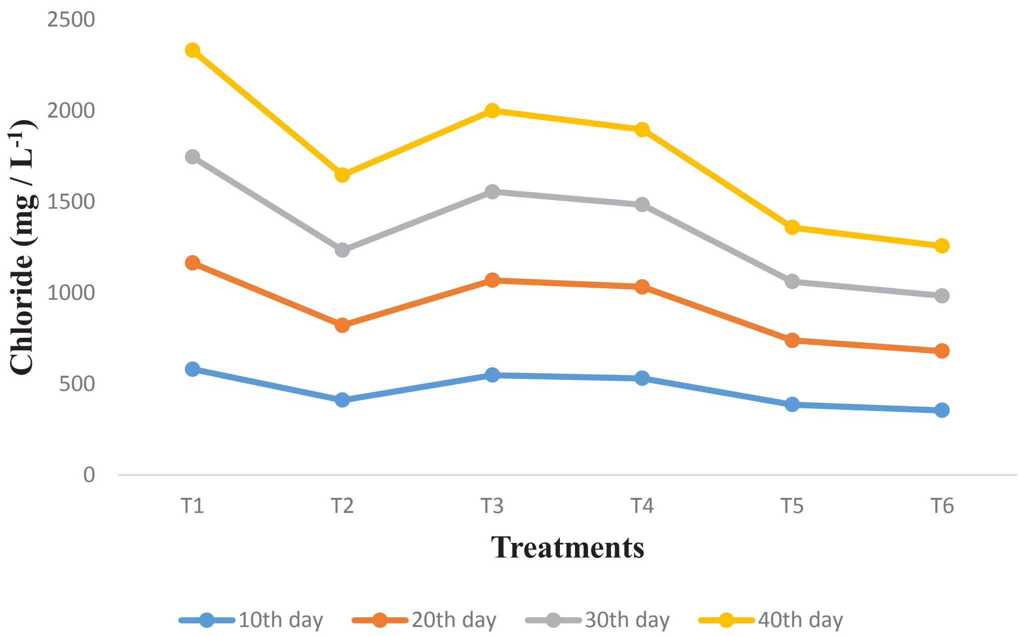

Fig 5. Effect of vetiver on Chloride (mg L^{-1}) content of paperboard mill wastewater

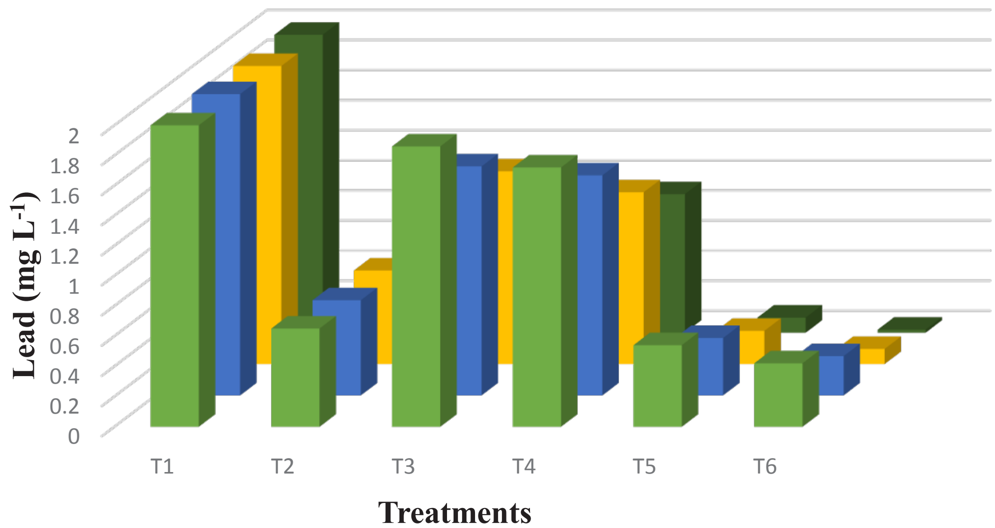

Fig 6. Effect of vetiver on Lead (mg L^{-1}) content of paperboard mill wastewater

The decrease in chloride content of papermill wastewater was noticed in all treatments with and without vetiver grass (Fig 5). The increase in chloride content was recorded in treatments (T_1 and T_2). The higher percentage (23.36 %) removal was recorded in T_6 (Treated effluent + Aeration + Vetiver system) followed by T_4 (Untreated effluent + Aeration + Vetiver system) recorded 18.27 % removal of chloride. The maximum content of chloride was 582.74 mg L^{-1} recorded in T_1 (Untreated effluent) and the minimum value (314.23 mg L^{-1}) recorded in T_6 (Treated effluent + Aeration + Vetiver system). The findings of present investigation is confirmed with Gerrard (2010) who reported that the accumulation of chloride was noted in vetiver root when it as used to treat the raw sewage wastewater and processed sewage wastewater.

Lead and cadmium

The treatments significantly influenced the lead and cadmium content of papermill wastewater (Fig 6 & 7). A slight decrease in lead and cadmium concentration was noticed in untreated and treated effluent alone. The lead content varied from 1.99 to 0.33 mg L^{-1}. The higher lead content was noticed (1.99 mg L^{-1}) in T_1 (Untreated effluent) and the lowest value (0.33 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The reduction percentage of lead was found to be high (65.63 %) in T_6 (Treated effluent + Aeration + Vetiver system). Among all the treatments, the cadmium content varied from 1.88 to 0.15 mg L^{-1}. The maximum cadmium content (1.88 mg L^{-1}) recorded in T_1 (Untreated effluent) and the minimum content (0.15 mg L^{-1}) was recorded in T_6 (Treated effluent + Aeration + Vetiver system). The reduction percentage of cadmium was recorded (64.29 %) in T_6 (Treated effluent + Aeration + Vetiver system). The reduction percentage of cadmium was higher than lead. The reduction percentage ranked in the order $T_6 > T_1$. This is supported by Liao et al. (2003) who opined that vetiver has ability to remove heavy metals from pig farm Nirola *et al*. (2016) revealed that the vetiver used to remediate heavy metal toxicity problems. Roongtanakiat *et al*. (2007) investigated with three industrial wastewaters *viz.,* battery manufacturing plant, electric lamp plant and ink manufacturing industr y wastewater and found that vetiver grass removed heavy metals (Pb, Fe, Mn, Zn, Cu) and in specific it has been removed maximum amount of lead than other heavy metals.

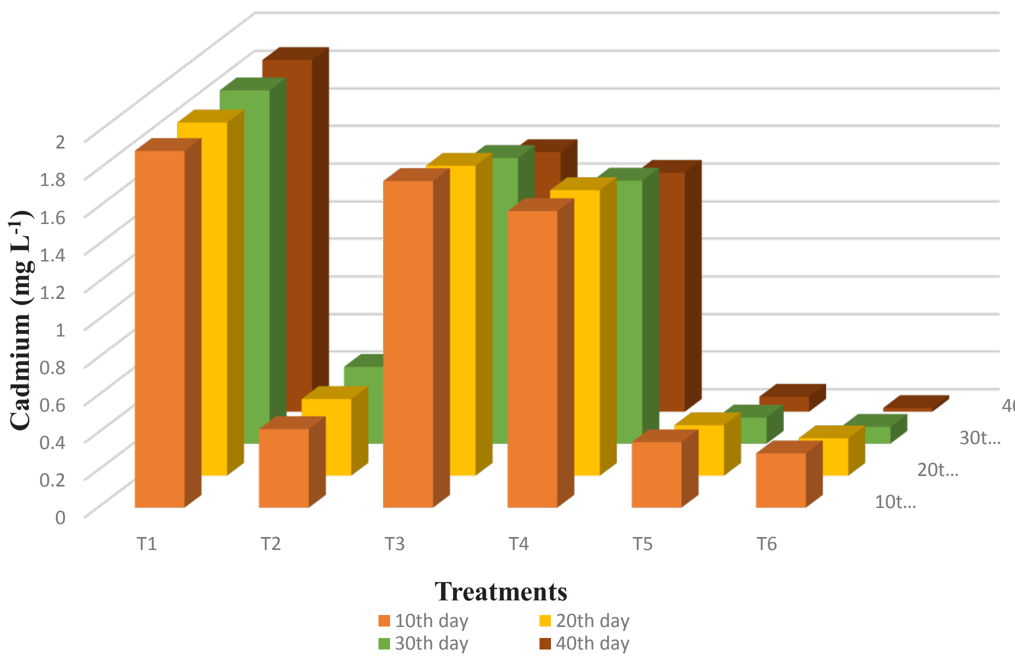

Fig 7. Effect of vetiver on Cadmium (mg L^{-1}) content of paperboard mill wastewater

Effect of paperboard mill wastewater on vetiver growth

Number of leaves

The number of leaves were increased in all treatments of the experiment (Table 6). Among the treatments the treatment T_6 (Treated effluent + Aeration + Vetiver system) recorded maximum number of leaves (20) and the minimum number of leaves (9) were recorded in T_3 (Untreated effluent + Vetiver system). This may be due to maximum uptake of nutrients and ions that contributes for the growth. This is in line with Dhanya and Jaya (2014) who reported that the number of leaves were increased in vetiver plant floated in sewage effluent than control. Darajeh *et al.* (2014) supplemented the statement by reporting that 86 number of leaves were recorded in vetiver plants after the treatment with low concentration palm oil mill effluent with in a period of two weeks.

Dry matter yield

The variable dry matter yield was recorded in different treatments (Table 6). The dry matter yield of shoot and root was found to be higher in T_6 (Treated effluent + Aeration + Vetiver system) (42.40 g, 45.70 g) followed by T_5 (Treated effluent + Vetiver system) (38.50 g, 40.70 g) and the lower dry matter yield was recorded in T_3 (Untreated effluent + Vetiver system) (20 g, 23.50 g). This might be due to the dry matter production is directly proportional to biometrics of vetiver grass. This is in line with the findings of Wagner *et al.* (2003) who supported the above findings by reporting that shoot dry matter yield of vetiver plants was upto 105 g / pot evidenced with the extreme uptake of nitrogen and phosphorus.

Effect of paperboard mill wastewater on proline

The proline content of vetiver is given in the table 7. The maximum proline content of shoot (3.49 microgram / gram) was recorded in the treatment of T_3 (Untreated effluent + Vetiver system) followed by T_4 (Untreated effluent + Aeration + Vetiver system) and the mimimum proline content (0.67 microgram / gram) was recorded in T_6 (Treated effluent + Aeration +Vetiver System). It might be due to stress condition enhanced by heavymetals *viz.,* Pb, Cd under untreated effluent. This is in line with the findings of Pang *et al.* (2003) who reported that the proline content was increased with increased heavy metal concentrations (pb) and it is also supported by Zhou and Yu (2010) opined that the proline content was increased which is directly proportional to stress condition.

Effect of paperboard mill wastewater on ELR

The ELR content of vetiver shoot and root were found to be high in untreated effluent than treated effluent (Table 7). In shoot, the highest percentage of ELR (93.04 %) was recorded in T_3 (Untreated effluent + Vetiver system) and the lowest percentage of ELR (77.52 %) was recorded in T_6 (Treated effluent + Aeration + Vetiver system), whereas, the highest percentage of ELR in root (78.81 %) was recorded in T_3 (Untreated effluent + Vetiver system) and the lowest percentage of 38.25 % was recorded in T_6 (Treated effluent + Aeration + Vetiver system). This might be due to the membrane damage which leads to the leakage of ions and it was found to be more in shoots than in roots. This is in line with Pang et al. (2003) shown that electrolyte leakage of both roots and leaves increased with the increased mine tailing concentrations (50%, 100%) at maximum treatment duration, but a significant increase was observed only for the 50 % and 100 % tailing treatments and the increase in the relative electric conductivity was more pronounced in shoots than in roots, implying that the injury of shoots was severer than that of roots.

Conclusion

Based on present investigation, it was obvious that hydroponically grown vetiver based treatment system with aeration significantly remove cations, anions, major nutrients and heavy metals from treated and untreated wastewater of paperboard mill.

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